IN THE CLAIMS

Claims 1-2 (canceled)

3. (original) An isolated complex comprising at least one ubiquitin or a derivative thereof, and a protein; wherein said protein is selected from the group consisting of aprataxin, tau, SLP, HMG17, PinX1, CIR, Cullin 3, HMGN3, HSPC144 and CDC6 and fragments and derivatives thereof; and said complex is formed via N-end rule ubiquitylation.

4. (previously presented) The complex of claim 1, wherein said complex is immobilized on a support and/or linked to a label.

5. (original) A method for producing a complex comprising at least one ubiquitin or a derivative thereof, and a protein; wherein said protein is selected from the group consisting of aprataxin, tau, SLP, HMG17, PinX1, CIR, Cullin 3, HMGN3, HSPC144 and CDC6 and fragments and derivatives thereof, comprising:

a) forming a mixture comprising a vector containing a clone coding for said protein, an *in vitro* transcription/translation system, an N-rule ubiquitylation system and, optionally, a proteasome inhibitor; and

b) incubating said mixture to allow production of said complex.

6. (original) The method of claim 5, further comprising:

c) isolating said complex.

7. (original) The method of claim 6, wherein said isolating is done by binding to an antibody specific to a poly-ubiquitin chain.

8. (original) The method of claim 6, wherein said isolating is done by binding to an antibody specific for said protein.

Claim 9 (canceled)

10. (previously presented) An isolated activated fragment of a protein, said fragment having an exposed N-degron, wherein said protein has a hidden N-degron and is selected from the group consisting of aprataxin, tau, SLP, HMG17, PinX1, CIR, Cullin 3, HMGN3, HSPC144 and CDC6 and fragments and derivatives thereof.

Claim 11 (canceled)

12. (previously presented) The activated fragment of claim 10, wherein said activated fragment is immobilized on a support and/or linked to a label.

Claims 13-14 (canceled)

15. (previously presented) A method of producing an activated fragment of a protein having an exposed N-degron, wherein said protein is selected from the group consisting of aprataxin, tau, SLP, HMG17, PinX1, CIR, Cullin 3, HMGN3, HSPC144 and CDC6 and fragments and derivatives thereof, comprising:

a) forming a mixture comprising said protein and a protease which cleaves said protein to form said activated fragment; and

b) incubating said mixture to allow production of said activated fragment.

16. (previously presented) The method of claim 15, further comprising:

c) isolating said activated fragment.

17. (original) An assay composition comprising an ubiquitin and a protein, wherein said protein is selected from the group consisting of aprataxin, tau, SLP, HMG17, PinX1, CIR, Cullin 3, HMGN3, HSPC144 and CDC6 and fragments and derivatives thereof and said ubiquitin and/or said protein is immobilized on a support and/or linked to a label.

Claims 18-24 (canceled)

25. (previously presented) A method for identifying at least one protease which cleaves a protein to expose an N-degron, the protein having a pro-N-degron and being selected from the group consisting of aprataxin, tau, SLP, HMG17, PinX1, CIR, Cullin 3, HMGN3, HSPC144 and CDC6 and fragments and derivatives thereof, comprising:

a) forming a mixture comprising said protein; and

b) screening a protease library for to identify proteases which i) bind said protein and/or ii) cleave said protein to expose said N-degron.

Claims 26-34 (canceled)

35. (previously presented) A method for identifying one or more active compounds that modulate N-end rule dependent ubiquitylation of a protein selected from the group comprising aprataxin, tau, SLP, HMG17, PinX1, CIR, Cullin 3, HMGN3, HSPC144 and CDC6, comprising:

a) forming a mixture comprising said protein or a fragment or derivative thereof, an N-rule ubiquitylation system, one or more candidate compounds and, optionally, a proteosome system;

b) measuring N-end rule ubiquitylation and/or proteosome-mediated degradation of said protein or a fragment or derivative thereof; and

c) identifying one or more compounds that modulate the rate of ubiquitylation or degradation.

36. (previously presented) The method of claim 35, wherein said protein or a fragment or derivative thereof includes a pro-N-degron and said mixture of step a) further includes a protease which exposes said N-degron.

37. (previously presented) The method of claim 35, wherein said protein or a fragment or derivative thereof is an activated fragment of said protein having an exposed N-degron.

38. (previously presented) The method of claim 35, wherein said active compound modulates activity of an E1 ligase, E2 ligase, E3 ligase, a protease that exposes said N-degron, or a combination thereof.

39. (previously presented) The method of claim 35, wherein said active compound modulates activity of an E1 ligase, E2 ligase and/or E3 ligase, or a combination thereof.

40. (original) A method for determining the mechanism of a compound that affects N-end rule ubiquitylation, comprising:

a) performing the identifying method of claim 36;

b) repeating said identifying method, except that said mixture further comprises an inhibitor of N-end rule ubiquitylation or said protein is replaced with a pre-activated fragment of said protein having said exposed N-degron; and

c) determining whether said compound is specific for said protease, and/or said N-end rule ubiquitylation system.

41. (original) A method for determining the mechanism of a compound that affects N-end rule ubiquitylation, comprising:

a) performing the identifying method of claim 35;

b) repeating said identifying method, except that said mixture further comprises an additional modulator of Type I, Type II and/or Type III N-end rule ubiquitylation; and

c) determining where said compound affects Type I, Type II and/or Type III N-end rule ubiquitylation.

42. (original) A method of making a pharmaceutical formulation containing one or more active compounds which modulate N-end rule ubiquitylation of a protein selected from the group consisting of aprataxin, tau, SLP, HMG17, PinX1, CIR, Cullin 3, HMGN3, HSPC144 and CDC6, comprising:

a) forming a mixture comprising said protein, or an activated fragment of said protein having an exposed N-degron, an N-rule ubiquitylation system, one or more candidate compounds and, optionally, a proteosome system;

b) detecting N-end rule ubiquitylation and/or proteosome-mediated degradation of said protein;

c) identifying one or more active compounds from said one or more candidate compounds; and

d) incorporating at least one of said one or more active compounds into a pharmaceutical formulation comprising said at least one active compound and suitable carrier.

43. (previously presented) The method of claim 42, wherein said one or more active compounds are inhibitors of N-end rule ubiquitylation.

44. (previously presented) The method of claim 42, wherein said one or more active compounds are promoters of N-end rule ubiquitylation.

45. (previously presented) The method of claim 42, wherein said one or more active compounds are naturally occurring.

46. (previously presented) The method of claim 42, wherein said one or more candidate compounds are selected from a compound library.

47. (previously presented) The method of claim 42, wherein said one or more candidate compounds are selected from a compound library of FDA approved drugs.

48. (previously presented) A method for modulating N-end rule ubiqutilation of a protein comprising administering one or more active compounds of claim 42.

49. (previously presented) A method for changing the level of a protein selected from the group consisting of aprataxin, tau, SLP, HMG17, PinX1, CIR, Cullin 3, HMGN3, HSPC144 and CDC6, comprising administering an effective amount of an active compound that modulates the rate of N-end rule ubiquitylation of said protein.

50. (original) A method of creating a modified protein by modifying a protein of interest to change its susceptibility to N-end rule degradation, said protein of interest selected from the group consisting of aprataxin, tau, SLP, HMG17, PinX1, CIR, Cullin 3, HMGN3, HSPC144 and CDC6, said method comprising:

i) decreasing the susceptibility of the protein to N-end rule degradation by modifying a protease cleavage site to prevent protease cleavage at said site, wherein cleavage at said protease cleavage site leads to exposure of an N-degron;

ii) increasing the susceptibility of the protein to N-end rule degradation by introducing a protease cleavage site for a known protease, wherein cleavage at said protease cleavage site leads to exposure of an N-degron; or

iii) decreasing the susceptibility of the protein to N-end rule degradation by modifying a protease cleavage site that when cleaved exposes an N-degron so that, after modification, the C-terminal product of said protease cleavage is not recognized by an N-end rule E3 ligase.

51. (original) The method of claim 50, further comprising expressing said modified protein in a cell.

52. (original) The method of claim 51, wherein the cell does not express the protein of interest.

53. (original) A method of generating a phenotypic cell line or animal, comprising:

a) generating a clone coding for a mutated form of a protein selected from the group consisting of aprataxin, tau, SLP, HMG17, PinX1, CIR, Cullin 3, HMGN3, HSPC144 and CDC6 and fragments and derivatives thereof, the mutated protein having a mutated protease cleavage site and/or N-degron and thereby modulating the susceptibility of the protein to N-end rule ubiquitylation; and

b) using said vector to transfect said cell line or to generate a transgenic animal by homologous or non-homologous recombination; and

c) detecting at least one phenotypic change relative to a control cell line or animal expressing the non-mutated form of the protein.

Claim 54 (canceled)

55. (original) A library of N-end rule ubiquitylation substrates including at least two proteins, or fragments or derivatives thereof, selected from the group consisting of aprataxin, tau, SLP, HMG17, PinX1, CIR, Cullin 3, HMGN3, HSPC144 and CDC6.

Claim 56 (canceled)